

## HCV X-Tail real time RT-PCR bench protocol

MasterMix:	µL
H2O	12.5
5X Reaction Buffer	10.0
dNTP (10mM each)	2.0
HCV-specific probe HCVMGB2	0.5
Internal Control-probe YFPY	0.5
Forward primer XTF5	1.0
Reverse primer HCMgR2	1.5
Enzyme Mix	2.0
<b>TOTAL</b>	<b>30.0</b>
	<b>+ 20.0 RNA</b>

\* all oligonucleotides are at 10µM concentration

\* dNTP, reaction buffer and enzyme mix from the Qiagen One-Step RT-PCR Kit (or similar)

\* RNA preparation with the Qiagen MinElute Spin Mini (or similar). If using a different extraction strategy, smaller amounts of RNA may be necessary to prevent from reaction inhibition.

\* Follow general PCR precautions like pipetting on ice, calculating 10% pipetting reserve etc.

### Oligonucleotide-Sequences:

XTF5	5'-GTGGCTCCATCTTAGCCCTAGT-3'
HCMgR2	5'-TGCGGCTCACGGACCTTT-3'
HCVMGB2	5'-FAM-CACGGCTAGCTGTG-MGBBHQ1-3'
YFPY	5'-VIC-ATCGTTCGTTGAGCGATTAGCAG-BHQ1-3'

### Thermal cycling program (ABI 7700 or similar):

Step	Time	Temperature (°C)	Repetition
Reverse Transcription (RT)	10 min	55	
Enzyme activation and RT inactivation	15 min	95	
PCR	10 sec	94	X 45
	20 sec <sup>a,b</sup>	58	

<sup>a</sup>On ABI 7000, 7300 and 7500 platforms a minimum annealing-extension time of 33 seconds at 58°C may be obligatory

<sup>b</sup>Fluorescence readout at this step

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For transfer of controls/standards:  
Please contact via email: [drexler@virology-bonn.de](mailto:drexler@virology-bonn.de)

**Requirements prior to shipment:**

1. Courier account number with confirmation of assumption of costs by recipient
2. Filled customs declaration forms as required in recipient's country

Shipment contents:

- 1 vial of plasmid template for quantification standard
- 1 vial of plasmid template for internal control

Please contact us separately if *in vitro*-transcribed RNA is requested